

The University of Chicago

AN EXPERIMENTAL ANALYSIS OF
HEMATOPOIESIS IN THE
RAT YOLK SAC.

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

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THREE PLATES (THIRTEEN FIGURES)

This report deals with attempts to modify embryonic hematopoiesis in rats, in order to study the potencies for development of the embryonic blood stem cells. At present no agreement has been reached concerning the identity of the embryonic stem cells of the mammalian yolk sac. Among the various theories the two extremes are in general represented by those who believe that the first free cell is endowed with the potentialities and functions of a complete hematopoietic stem cell (that is, a hemocytoblast) and the contrary view which holds that this first free cell is a primitive erythroblast, a cell unalterably oriented solely into the direction of hemoglobin carriage. The various views are considered in many of the chapters of Downey's Handbook of Hematology.

One reason for this divergence of opinion may be the use of different techniques. In general, the users of the section technique are proponents of the first theory, while the second theory is based primarily on the results obtained with the dry smear technique. Kirschbaum and Downey ('37) have attempted to correlate the results of both techniques. According to them, the dry smear technique is superior to the section technique. They believe that the first free blood cell of the rat yolk sac is a primitive erythroblast.

¹ The substance of this report was offered as a thesis in partial fulfillment of the requirements of the degree of Doctor of Philosophy, Department of Anatomy, The University of Chicago.

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I have undertaken to investigate the identity of this first free blood cell of the rat yolk sac by the use in parallel of dry smears and sections and have also added an experimental approach to the problem. By subjecting the blood cells to various stimuli certain developmental potencies, not realized normally, come into appearance and give further information about the identity of the cells.

MATERIALS AND METHODS

To obtain embryos of known age male rats were mated to normal females in the evening, and the next morning vaginal smears were made. The morning after finding a positive smear was counted as the end of the first day of pregnancy. All tissues were fixed for 2 to 6 hours in formol-Zenker solution (Maximow). Embryos younger than 16 days were fixed after the yolk sac and amnion were incised. The membranes from older embryos were separated from the body before fixation.

The tissues were embedded in nitrocellulose, cut serially at 8μ , and stained with hematoxylin-eosin-azure II and Mallory-azan. Some slides were also impregnated by the Bielschowsky silver technique. The dry smears were stained with May-Grünwald Giemsa and toward the end of the work by the Kingsley stain.

Attempts were made to influence hematopoiesis in the rat yolk sac by subjecting pregnant rats to the following experimental procedures. Pregnant rats were made anemic by injection with sapotoxin, phenylhydrazine, sulfanilamide, or dinitrophenol and by repeated bleeding. Thyroid extract and dinitrophenol were injected into pregnant rats in attempts to accelerate maturation of the blood cells of the embryos by initiating an earlier formation of the embryonic definitive erythroblasts. Because of their efficacy in converting the megaloblastic bone marrow of pernicious anemia to a normoblastic bone marrow, normal human gastric juice and liver extract were also administered to pregnant rats on the chance they might affect the primitive erythroblasts of the embryos.

Chloroform by inhalation and Evan's blue by injection were used because of their high placental permeability.

In addition, intra-ocular transplants of the yolk sac were made because of the possibility that the realization of unusual potentialities of the embryonic blood cells might be elicited by the changed environment. It was further hoped that in the absence of the placenta, these grafts might be more susceptible to stimuli affecting the mother rats.

The following paragraphs list the experiments in detail:

Sapotoxin. One-half cm^3 of a 0.5% solution was injected intraperitoneally three times between the sixth day of pregnancy and autopsy. 13½-, 14½- and 16-day embryos were studied.

Phenylhydrazine. One-half cm^3 of a 0.5% phenylhydrazine solution was injected daily beginning with the fifth day of pregnancy. 11-, 12-, and 16-day embryos were studied.

Sulfanilamide. One-fourth gm of sulfanilamide per kilogram of rat was mixed with the food and fed daily beginning on the fifth day of pregnancy. Higher doses caused death of the fetuses. 11-, 16-, and 21-day embryos were studied.

Blood loss. Blood was removed by sterile heart puncture from the pregnant rats. Three embryos were studied: one of 10½ days whose mother had lost 13 cm^3 of blood during pregnancy, another of 13½ days whose mother had lost 13½ cm^3 during this period, and a 15-day embryo whose mother had lost 40 cm^3 during this period and the preceding month. In the last case the 15 cm^3 taken during pregnancy were replaced by an equal volume of 0.9% NaCl solution. The blood was withdrawn at irregular intervals, so spaced as not to kill the pregnant rats.

Dinitrophenol. A solution of 1 gm of dinitrophenol and 0.5 gm of Na_2CO_3 in 100 cm^3 of water was injected intraperitoneally in 1.5 cm^3 doses. An exception was the case of a 9-day embryo whose mother received 2 cm^3 doses. Each animal was injected daily, beginning the second or third day of pregnancy. 9-, 14-, and 18-day embryos were studied.

Thyroid extract. From the fourth day of pregnancy until autopsy on the twelfth day, a rat received 0.075 gm daily of thyroid extract mixed in the food. Another rat received 0.75 gm daily in the food until autopsied on the seventeenth day.

Gastric juice. Normal human gastric juice was obtained by Rehfuess tube, concentrated to one-tenth its volume by a fan or by heating at 37°C., filtered, neutralized by N/10 NaOH and stored on ice until ready for use. Two cm³ were injected intraperitoneally daily during pregnancy. 11-, 14-, and 17½-day embryos were studied.

Lederle liver extract. One cm³ of the "concentrated parenteral solution" was injected intramuscularly on the third day of pregnancy; 12- and 13-day embryos were studied.

Evan's blue. Four mg of Evan's blue in 1 cm³ of water were injected intraperitoneally into each of two rats on the eighth day of pregnancy. These were killed 6 days later. Four mg were injected on the eighth and on the fourteenth day of pregnancy into another rat which was killed 4 days later.

Chloroform. Six pregnant rats were maintained under first stage anesthesia for 15 minutes daily beginning with the sixth day of pregnancy. One 10-day embryo was obtained for study; the others died.

Grafts. Pieces of yolk sac from 11- to 17-day rat embryos were grafted aseptically into the anterior chamber of the eye of adult rats. Fifty-two grafts were recovered when the receptors were killed at intervals of 1 to 30 days after implantation. The grafts were fixed in formol-Zenker, sectioned in nitrocellulose at 8 μ and stained with hematoxylin-eosin-azure II.

Another series of twenty-five grafts of yolk sac of 11-, 15- and 16-day embryos was transplanted in the same way into rats receiving 2 to 3 cm³ of a 10% solution of sulfanilamide in propylene glycol by stomach tube. The treatment was begun 3 weeks before transplantation and maintained at 3 cm³ daily, until the animals became very sick, when the dose was cut down or omitted entirely. Most of these rats received an

average daily dose of $2\frac{1}{2}$ cm³. In these animals the grafts were maintained for periods varying from 1 to 30 days.

OBSERVATIONS ON THE PREGNANT RATS

The liver extract and gastric juice did not affect visibly the pregnant rats. Blood smears from the tails of those rats injected with sapotoxin, phenylhydrazine, sulfanilamide and dinitrophenol showed basophilia of the erythrocytes and pathological granulation of the leukocytes. As a result of chronic blood loss occasional normoblasts appeared in the smears. The Evan's blue colored the maternal tissues deep blue.

Thyroid extract and dinitrophenol made the pregnant rats very nervous and possibly increased the percentage of resorbed embryos. The animals fed sulfanilamide in propylene glycol had a great deal of trouble walking immediately after the treatment, and during the experiment developed distended abdomens and became very lethargic.

OBSERVATIONS ON THE EMBRYOS

None of the experiments produced any significant histological changes in the embryos. The Evan's blue was stored in the yolk sac epithelium (fig. 11), but did not influence the hematopoiesis. The following observations hold true in both the normal and the experimental animals.

Yolk sac. The extensive migration of the mesoderm into the yolk sac begins at about the ninth day. At this stage the cells of the mesoderm have much the same appearance as the adjoining endodermal cells. Both tissues are composed of closely packed cells with a distinctly vacuolated basophilic cytoplasm and large irregular nucleoli. The mesoderm develops into mesenchyme which shortly gives rise to blood island formation. A few mesenchymal cells remain unchanged outside the blood islands. At this stage, free basophil cells are formed from the more centrally located cells of the blood islands, and, to a far lesser extent, from the lining cells of the sinuses derived from the periphery of the blood islands.

The dry smears and sections were compared in order to study the identity of these free basophile cells. Up to about 13 days, cells corresponding morphologically to hemocytoblasts are found regularly in both sections and dry smears (figs. 5, 6, 8). In the sections these cells are intravascular.

In dry smears the hemocytoblast in the yolk sac has all the characteristics of this cell type as found in dry smears of the bone marrow of the adult rat and the embryonic liver of the rat (compare fig. 7 with fig. 8). In this material the hemocytoblast has a large nucleus composed of very finely punctate chromatin with poorly defined nucleoli and a narrow rim of basophil cytoplasm. It resembles very closely some of the ectoplacental cells, but the latter have a more stringy, coarser chromatin distribution and a very poorly defined cytoplasmic border, in my opinion, much like the yolk sac "hemohistoblast" illustrated by di Guglielmo ('22) and Ferrata ('33-'35). Even in the youngest stages (10 days) the hemocytoblasts are few in number and decrease steadily with age. Although regularly present, the cell has to be diligently searched for in the dry smears. In the sections which were studied serially through the entire length of the embryo, hemocytoblasts (figs. 5 and 6) average about four or five cells in every section of the yolk sac of a 12-day rat embryo.

In both sections and smears the hemocytoblast must be discriminated from the basophilic primitive erythroblast into which it develops gradually. In sections the breakup of the nucleoli, or the appearance of irregular chromatin masses, or the deepening of the cytoplasmic basophilia was considered evidence of the beginning transformation of the hemocytoblast into the primitive basophilic erythroblast (figs. 3 and 4). In smears the first sign of the coarsening of the chromatin or its change from a punctate to a cord-like arrangement (Ferrata and De Negreiros Rinaldi, '14; Jones, '38), or the deepening of the cytoplasmic basophilia was accepted as indicative of the change to the primitive basophil erythroblast (fig. 10). After this time (12 to 13 days) I could not regard the remaining hemocytoblasts in the yolk sac sinuses as being exclu-

sively of local origin because they are also found in the liver (fig. 7) and might possibly be transported from there into the yolk sac sinuses. However, I have no evidence that this occurs.

On the eleventh-twelfth day of pregnancy, as seen in sections, some of the extravascular mesenchymal cells begin to contract, the nuclei stain darker owing to a clumping of the chromatin and a thickening of the nuclear membrane, and a rather large irregular acidophil nucleolus forms. The cytoplasm becomes basophilic and develops fine vacuoles with the retraction of the processes, and becomes sharply demarcated. A pale centrosphere is usually seen. By the thirteenth or fourteenth day these rounded cells are recognizable as hemocytoblasts identical in appearance with the previously described intravascular hemocytoblasts. They then develop into definitive basophil erythroblasts, increase mitotically, and as a result are often found in pairs. Occasionally some of the cells are found inside endodermal cells (fig. 11). This process continues until about the eighteenth day when relatively large masses of definitive erythroblasts are found extravascularly in the yolk sac. Previous workers have failed to report or have denied the presence of definitive erythroblasts and myelocytes in the rat yolk sac (Maximow, '09, '27; Bloom, '38; Kirschbaum and Downey, '37; Jones, '41).

The formation of myelocytes occurs extravascularly beginning about the thirteenth day in a similar manner, but the stem cell is usually a histioid wandering cell, less often a hemocytoblast (fig. 13). The process is much the same as in the formation of the first myelocytes in the loose mesenchyme of the body and in the earliest stages of the development of the embryonic bone marrow (Maximow, '10). The mesenchymal cell withdraws its processes, the chromatin stains very heavily, the nuclear membrane becomes thick, the nucleus becomes slightly indented, and the cell is now recognizable as a histioid wandering cell. In the event that an eosinophil myelocyte is being formed, granules appear in the cytoplasm, the nucleus becomes lobate, and the chromatin splits into small dark

masses suspended in a linin network (fig. 12). The myelocytes increase by mitotic proliferation. They are spread diffusely through the perivascular mesenchyme and do not form clumps with the more mature cells peripherally, as do the definitive erythroblasts.

The first heterophil myelocytes are formed a day or two later. In the rat the heterophil granulation cannot usually be seen in sections even with the highest power of the microscope, but the cytoplasm has a distinctly pinkish tint with hematoxylin-eosin-azure II. The transformation of the histioid wandering cell into the heterophil myelocyte is marked by the same nuclear changes as occur in the formation of the eosinophil myelocyte, but granules rarely appear. Instead the cytoplasm turns a diffuse pink, which gradually becomes more pronounced. The heterophils always appear less numerous than the eosinophils. This may be due in part to the greater difficulty of recognizing the heterophils since they lack the brilliant eosinophilic granules.

Megakaryocytes are also formed intravascularly in exceedingly small numbers. They are derived from the hemocyto-blasts by a gradual increase in lobation of the nucleus as well as an increase in size. Occasionally the primitive erythroblasts also undergo a lobation of their nuclei, but apparently do not become megakaryocytes. These changes of the primitive erythroblasts were especially noticeable in a degenerating 11-day embryo. The cytoplasm of these atypical primitive erythroblasts contains vacuoles and is homogeneously blue with a small attraction sphere and so may be distinguished from the cytoplasm of the megakaryocyte. Also the nuclei are different: the chromatin of the megakaryocyte is not arranged in irregular bars and clumps as in the degenerating erythroblasts, but is distributed in the form of small angular masses suspended in a very faint linin network.

By the fourteenth day numerous reticular fibers are present. In Bielschowsky impregnations, and to a lesser extent in slides stained with the Mallory-azan method, these fibers form a very distinct mesh-work about the vessels. A particularly

heavy condensation occurs at the base of the endoderm, forming a basement membrane. In hematoxylin-eosin-azure II sections there is a large, distinctly eosinophilic fiber running adjacent to the mesothelium. In the silver impregnated sections it is present as a dense mass of reticular fibers.

By about 18 days the myelocytes and the basophil definitive erythroblasts, some of which have now become polychromatophil and eosinophil definitive erythroblasts have formed large extravascular masses of cells extending parallel to the vessels. Sometimes the clumps are so large that they begin to bulge into the sinuses. Large masses of myeloid cells are found projecting from the perivascular tissue in the villus-like projections into the sinuses along the mesothelial border of the yolk sac.

Intra-ocular grafts of yolk sac. The yolk sac of the rat embryo is made up essentially of sinuses filled with large numbers of primitive erythroblasts in various stages of maturation depending on the age of the embryo. Outside of the sinuses are found a few scattered mesenchymal cells at about 11 and 12 days and later a few foci of developing definitive erythroblasts and myelocytes derived from some of these mesenchymal cells. Although the process of definitive erythropoiesis and myelocytopoiesis has been described at length because of its great theoretical value, the definitive erythroblasts and myelocytes numerically represent an inconsequential percentage of the yolk sac cells.

But in all the intra-ocular yolk sac grafts this quantitative relationship changes so intensely that one might think of it as almost a qualitative difference between the yolk sac hematopoiesis in the normal environment and in the anterior chamber of the eye. This change occurs in essentially a similar manner in all the grafts, regardless of the state of development of the yolk sac at the time of transplantation, that is from 11 to 17 days. During the first 2 or 3 days the grafts are being vascularized and the degeneration and debris incident to the transplantation itself is being cleared. By the third or fourth day the graft is vascularized.

One of the earliest and most spectacular changes in the grafts is the complete disappearance of the primitive erythroblasts so that by 3 to 4 days after transplantation none are to be found. During these 3 to 4 days mitoses of these cells are extremely rare in contrast to the almost continuous mitotic proliferation of these cells in their normal environment. The primitive erythroblasts decrease so quickly in number that the mechanism of their disappearance, except for the cessation of mitoses, is not clearly evident.

Beginning at the second to fourth day after transplantation, large numbers of definitive erythroblasts, megakaryocytes, and myelocytes develop from the mesenchymal cells of the yolk sac. This process is qualitatively similar to that described previously in the extravascular tissues of the normally developing yolk sac, that is, the mesenchymal cells contract down to form hemocytoblasts which in turn give rise to definitive erythroblasts, myelocytes and megakaryocytes. Also, erythroblasts and myelocytes undergo mitotic proliferation. There is no evidence of any relationship between the primitive erythroblasts originally present in the yolk sac sinuses at transplantation and the definitive erythroblasts which appear in the grafts after about 4 or 5 days. The latter first appear as basophilic erythroblasts directly related in location and transitional forms to hemocytoblasts at a time when the few remaining primitive erythroblasts have all become eosinophilic. Occasionally, the mesenchymal cells may develop directly into eosinophil myelocytes, as evidenced by the appearance of specific granulation in the mesenchymal cells. About 16 days after transplantation the grafts are gradually replaced by or become loose connective tissue.

A slightly smaller number of myelocytes and definitive erythroblasts develops in the grafts transplanted into animals treated with sulfanilamide. This difference is far less marked than the difference between hematopoiesis in the grafts and the embryo.

From the beginning of the existence of the grafts large numbers of lymphocytes and heterophil leukocytes migrate in

from the blood vessels of the host and large numbers of exudate mononuclears develop. The interpretation of the relationship between these inflammatory cells and the hematopoiesis in the grafts requires further study.

DISCUSSION

Many of the experimental stimuli used in this work have been previously shown to affect post-natal hematopoiesis. Of these, sulfanilamide (Speert, '40, in rat; Barker, '38, in man), chloroform (Adair, '41, in man) and thyroid extract (Grumbrecht and Loeser, '38, in rat), have been demonstrated in the fetal tissues after exposure of the pregnant female to the drug. Nevertheless, none of the substances used, some of which are very toxic to post-natal animals, was able to affect embryonic hematopoiesis. It was only when the embryonic tissues were transplanted to the anterior chamber of the eye that extreme changes in the normal pattern of hematopoiesis resulted.

Because of the resemblance of some aspects of the blood picture of the fetus to changes found in pernicious anemia patients subjected to an adequate stimulus to blood formation, Wintrobe and Schumacker ('35) suggested that the transference of the anti-anemic principle from the mother to the fetus is the cause for the normal developmental changes in the blood picture of the embryo. Acting upon this theory a long series of experiments has been performed upon rat embryos in which liver extract, ventriculin or gastric juice were introduced into the pregnant females with the hope of accelerating the appearance or maturation of the definitive erythroblasts and of using these changes to assay the drugs. Rats were usually chosen primarily because of the supposed lack of definitive erythrocytopoiesis in the rat yolk sac. These experiments, which have given contradictory results in the hands of several investigators, are reviewed by Jones ('41) and Last and Hays ('42) and Hays and Last ('42).

All investigators have admitted the extreme normal variation in the size and shape of the embryonic rat's erythrocytes. The peripheral blood of the embryo does not reflect accurately

the picture in the blood-forming organs. In a histological study of the rat embryo by the use of complete serial sections I was unable to find that injection of gastric juice or liver extract into the pregnant rat modifies hematopoiesis in the embryos.

The nature of the first free basophil cell of the yolk sac has been the subject of much controversy. Schridde ('07), Naegli ('31) and Amano, Hagio and Kyo ('39) have claimed that the first free blood cell of the yolk sac is a primitive erythroblast. Schridde's results, as well as those of Amano, Hagio and Kyo have been based on sections of human yolk sacs. Knoll ('32), Bloom ('38), Bloom and Bartelmez ('40), and Gladstone and Hamilton ('41) have found hemocytoblasts in human yolk sacs. Kirschbaum and Downey ('37), using dry smears of rat yolk sacs, found only primitive erythroblasts and no hemocytoblasts. In my dry smears of the rat yolk sac, besides the primitive erythroblasts a few cells are found which are identical with the hemocytoblast (myeloblast of Downey, '27, '38) found in dry smears of the liver of rat embryos.

The development of the hemocytoblast can be traced from the mesenchyme of the yolk sac in sections of 9- to 10-day embryos. The hemocytoblasts then begin to form primitive erythroblasts and decrease sharply in number. Primitive erythroblasts, a few endothelial phagocytes and rarely megakaryocytes are the only cells formed intravascularly in the rat yolk sac. By the eleventh day the intravascular hemocytoblasts are so rare that the yolk sac sections must be studied serially to demonstrate them, but they can always be found. The intravascular hemocytoblasts are present in fairly large numbers only in that brief interval between their formation in the blood islands and their rapid and almost complete conversion into and replacement by the primitive erythroblast. At this time the embryo and its membranes are very small, measuring about 1 mm in length (Henneberg, '37). The maximum diameter of the yolk sac sinuses is about 30 to 40 μ . The most immature cells invariably occur in the smallest sinuses so that it is difficult, in a dry smear, to obtain these cells in an undamaged

condition and free from overlying or adherent cells. The hemocytoblasts are likely to be missed and only the more mature cells from the less densely populated larger sinuses, the primitive erythroblasts, will be found in dry smears. In spite of an intensive study of the yolk sac dry smears, I have never been able to find any sign of the formation of definitive erythroblasts and myelocytes which is so clearly demonstrated in the sections of the yolk sacs of litter mates of these rat embryos (figs. 11 and 12).

It must be emphasized that the differences between the hemocytoblast and the most immature primitive and definitive erythroblasts, as well as the differences between the latter two, are very slight and of such a nature as to be interpreted differently by even the most experienced hematologists. When one compares these cells in the illustrations of Downey ('27), Naegli ('31), Ferrata and de Negreiros-Rinaldi ('14), Ferrata ('33-'35) and Jones ('38), it is apparent that there is as much variation among the individual hemocytoblasts (myeloblasts or stem cells) as between the hemocytoblasts and the most immature erythroblasts. Since the stem cells differentiate imperceptibly into the primitive or definitive erythroblast, I believe there must necessarily be a point, no matter how the cells are prepared, at which the cells will be sufficiently similar to each other to defy differentiation on purely morphological grounds. A study of the potentialities of the cells in question must be resorted to in order to supplement the purely morphological approach.

Therefore, in order to prove that the first free basophilic cell of the yolk sac is a hemocytoblast, one must show that this cell is potentially as well as morphologically a hemocytoblast. This cell is the rat yolk sac in situ develops primarily into primitive erythroblasts, a few phagocytes and rarely megakaryocytes intravascularly and into only a few definitive erythroblasts, eosinophils and heterophils extravascularly. However, when the rat yolk sac is transferred into the anterior chamber of the eye, this same cell produces great numbers of definitive erythroblasts, granulocytes and megakaryocytes

and ceases to produce primitive erythroblasts. It should be noted, moreover, that in the yolk sac of many other mammals it forms definitive erythroblasts, heterophils, eosinophils and various types of giant cells (Maximow, '09, '27; Kiyono and Nakanoin, '19; Jolly, '23; Bloom, '38; Bloom and Bartelmez, '40). A similar condition exists in the chick yolk sac where this stem cell forms erythroblasts for the most part but when it is grafted onto the chorio-allantoic membrane it will develop mainly into granulocytes (Dantschakoff, '24). These experimental observations substantiate the view that the first free blood cell of the yolk sac of the rat is a free blood stem cell (hemocytoblast) since it has the potentiality of forming not only primitive erythroblasts but also definitive erythroblasts, granulocytes and megakaryocytes.

CONCLUSIONS

1. Although toxic stimuli may seriously disturb hematopoiesis in pregnant rats, hematopoiesis in the embryos remains unaltered.

2. Intra-ocular transplantation changes the hematopoietic processes in the embryonic yolk sac. The administration of large amounts of sulfanilamide to rats with these intra-ocular transplants makes only an insignificant change in the development of these grafts.

3. There is little reason to believe that the rat embryo is suffering from a lack of anti-anemic principle. Feeding this principle to pregnant rats does not influence embryonic hematopoiesis.

4. Hemocytoblasts (myeloblasts), as well as most of the bone marrow cells of the adult are found in the rat yolk sac.

5. Serial sections stained with hematoxylin-eosin-azure II are more satisfactory than dry smears for the study of embryonic hematopoiesis.

6. Regardless of the cytological techniques used, the morphology of the cells is insufficient to characterize them completely. The question of their potentialities for development

must be probed before conclusions on their exact nature can be reached.

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PLATES

EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida at table level and with a Zeiss 2 mm, 1.4 NA apochromatic objective and compensating oculars.

PLATE 1

EXPLANATION OF FIGURES

Figures 1-6 are from sections of a 12-day rat embryo yolk sac, formol-Zenker fixation, hematoxylin-eosin-azure II, 1700 $\times$.

- 1 Polychromatophil primitive erythroblast.
- 2-4 Basophil primitive erythroblasts.
- 5-6 Hemocytoblasts.

Figures 7-10 are from dry smears, May Grünwald-Giemsa, 1700 $\times$.

- 7 Hemocytoblast from the liver of a 16-day embryo.
- 8 Hemocytoblast from the yolk sac of a 12-day embryo.
- 9-10 Basophil primitive erythroblasts from yolk sac of a 12-day embryo; 9 more mature than 10.

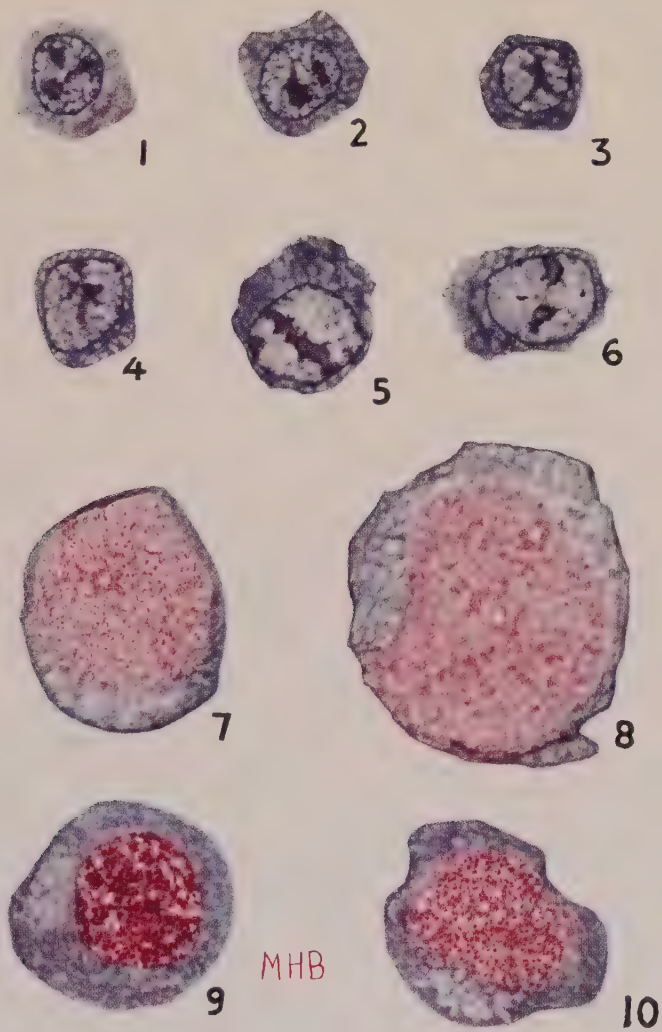


PLATE 2

EXPLANATION OF FIGURES

Figures 11-12 from yolk sac, formol-Zenker formation, hematoxylin-eosin-azure II, 2700 X.

- 11 Sixteen-day embryo, definitive erythroblasts within endodermal cells.
- 12 Thirteen-day embryo, extravascular formation of eosinophil myelocytes.

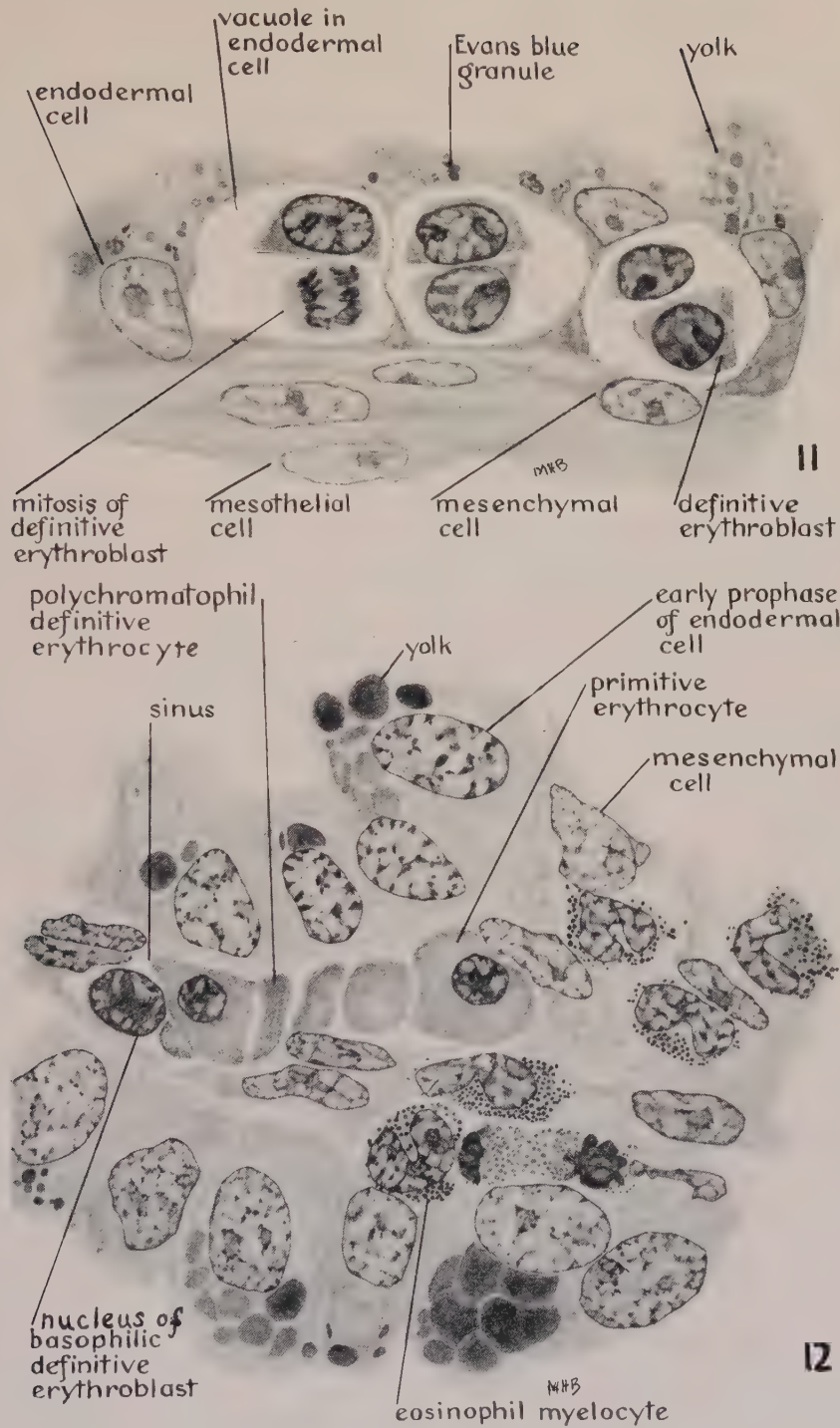


PLATE 3

EXPLANATION OF FIGURES

13 Thirteen-day embryo, yolk sac, formol-Zenker fixation, hematoxylin-eosin-azure II, 2700 $\times$.

a. Transition from mesenchymal cell to histioid wandering cell.



